EXHIBIT C

1: Biochim Biophys Acta. 1987 Aug 7;902(1):24-30. Related Articles, Links

Single mutation in the A domain of diphtheria toxin results in a protein with altered membrane insertion behavior.

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The insertion of the A domain of diphtheria toxin into model membranes has been shown to be both pH- and temperature-dependent (Hu and Holmes (1984) J. Biol. Chem. 259, 12226-12233). In this report, the insertion behavior of two mutant proteins of diphtheria toxin, CRM197 and CRM9, was studied and compared to that of wild-type toxin. Results indicated that both CRM197 and CRM9 resembled toxin with respect to the pH-dependence of binding to negatively-charged liposomes at room temperature. However, CRM197 differed from toxin with respect to both the pH- and temperature-dependence of fragment A insertion; fragment A197 inserts more readily into the bilayer at 0 degrees C and low pH or at neutral pH and room temperature than does wild type fragment A under these same conditions. This result indicates that the single amino acid substitution in the A domain of CRM197 facilitates entry of fragment A197 into the membrane, suggesting that CRM197 may be conformationally distinct from native toxin. In fact, the fluorescence spectra of CRM197 and wild-type toxin as well as their respective tryptic peptide patterns indicate that, at pH 7, CRM197 more closely resembles the acid form of wild-type toxin than the native form of toxin. These data suggest that CRM197 may be naturally in a more 'insertion-competent' conformation. In contrast, the mutation in the B domain of CRM9 which results in a 1000-fold decrease in binding affinity for plasma membrane receptors apparently does not cause a change in either the insertion of fragment A9 or the lipid-binding properties of CRM9 relative to toxin.

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